Intracellular diffusion in the presence of mobile buffers

Application to proton movement in muscle

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ABSTRACT Junge and McLaughlin (1987) derived an expression for the apparent diffusion constant of protons in the presence of both mobile and immobile buffers. Their derivation applies only to cases in which the values of pH are considerably greater than the largest pK of the individual buffers, a condition that is not expected to hold in skeletal muscle or many other cell types. Here we show that, if the pH gradients are small, the same expression for the apparent diffusion constant of protons can be derived without such constraints on the values of the pK's. The derivation is general and can be used to estimate the apparent diffusion constant of any substance that diffuses in the presence of both mobile and immobile buffers. The apparent diffusion constant of protons is estimated to be 1–2 × 10^{-6} cm^2/s at 18°C inside intact frog twitch muscle fibers. It may be smaller inside cut fibers, owing to a reduction in the concentration of mobile myoplasmic buffers, so that in this preparation a pH gradient, if established within a sarcomere following action potential stimulation, could last 10 ms or longer after stimulation ceased.

INTRODUCTION

The diffusion of ions and small molecules inside cells is generally slower than that in simple aqueous solutions. In some cases, this is due to binding of the ions or molecules to relatively immobile intracellular components, such as membrane-bound organelles or cytoskeletal proteins. If this binding is linear and reversible, so that the concentration of the bound molecules is always equal to that of the free molecules times a constant (R), then, according to Crank (1956), the apparent diffusion constant is $D/(1 + R)$, in which D is the free diffusion constant in cytoplasm (which might be lower than that in aqueous solution because of physical factors such as a higher viscosity). Ions and small molecules may also be bound to intracellular components that are mobile; for example, protons may be bound to small diffusible buffer molecules. This kind of binding can also influence the apparent diffusion constant.

An expression for the apparent diffusion constant of protons in the presence of both mobile and immobile buffers was derived by Junge and McLaughlin (1987), under the assumption that the largest pK of the individual buffers is considerably less than the value of intracellular pH. This corresponds to the assumption that binding is linear and nonsaturating, similar to the assumption used in the treatment of diffusion in the presence of immobile binding sites referenced above. In many cases of biological interest, however, binding is not linear and these simplifying assumptions are not valid.

In this article, we use a different assumption to derive expressions for the apparent diffusion constant of any molecule in the presence of both mobile and immobile buffers (or binding sites): we assume that the changes in concentration of the molecule are relatively small. The expressions for the apparent diffusion constant derived here are essentially the same as those derived by Junge and McLaughlin (1987). In the final section of this article, these expressions are used to estimate the apparent diffusion constant of protons in frog twitch muscle, 1–2 × 10^{-6} cm^2/s at 18°C.

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RESULTS AND DISCUSSION

Theory

To determine the apparent diffusion constant of a substance S inside a cell, the intracellular buffers of S are separated, conceptually, into those that are mobile, and consequently free to diffuse, and those that are immobile,

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or fixed. $[B_n]$ represents the total concentration (i.e., free plus bound to S) of each of the N species of mobile buffers, $1 \leq n \leq N$. If a_n represents the average number of molecules of S that are bound per molecule of B_n, the concentration of S that is bound by B_n, $[S_n]$, is given by

$$[S_n] = a_n[B_n]. \tag{1}$$

The properties of the immobile buffers may be represented by a single concentration $[B_0]$ and a factor a_0 chosen so that the total concentration of S bound by immobile buffers, $[S_0]$, is equal to $a_0[B_0]$. a_n is assumed to be a function of $[S]$ only and not to depend directly on either time or location within the cell or on $[B_n]$. Buffer reactions are assumed to be fast and in equilibrium at all times.

For each mobile buffer, the free diffusion constant, D_n, is assumed to be constant throughout the cell and independent of how many molecules of S are bound. The movement of each buffer is assumed to obey the diffusion equation, which, for isotropic media, can be written

$$\partial[B_n]/\partial t = D_n\nabla^2[B_n], \tag{2}$$

$1 \leq n \leq N$. The corresponding equation for S is

$$\partial[S]/\partial t + \sum_{n=0}^{N} \partial[S_n]/\partial t = D_S\nabla^2[S] + \sum_{n=1}^{N} D_n\nabla^2[S_n]. \tag{3}$$

D_S is the diffusion constant of S. In the absence of sources and sinks for buffers, $[B_n]$ is constant, $0 \leq n \leq N$, and Eqs. 1 and 3 give

$$\partial[S]/\partial t + \sum_{n=0}^{N} [B_n]\partial a_n/\partial t = D_S\nabla^2[S] + \sum_{n=1}^{N} D_n[B_n]\nabla^2 a_n. \tag{4}$$

An expression for D_S^{app}, the apparent diffusion constant of S, can be easily obtained from Eq. 4 if a_n is directly proportional to $[S]$, a condition that holds only when $[S]$ is considerably less than any of the dissociation constants of the individual buffers. In the case of proton diffusion, this condition requires that the intracellular pH be considerably greater than the pK's of all the buffers. Junge and McLaughlin (1987) derived an expression for D_H^{app} for this case, which applies to both small and large gradients of pH.

In many situations, however, the condition that $[S]$ is considerably less than any of the dissociation constants of the individual buffers does not apply. For example, in the case of proton diffusion in muscle, which is considered in detail below, some of the buffers have pK's near the value of myoplasmic pH (Godt and Maughan, 1988), so that the direct proportionality between a_n and $[H^+]$ assumed by Junge and McLaughlin (1987) is not expected to hold. Nonetheless, an expression for D_S^{app}, similar to their expression for D_H^{app}, can still be derived for the case in which the gradients of $[S]$ are small.

For our derivation, $[S]$ and a_n are written

$$[S] = [S(\infty)] + \Delta[S], \tag{5}$$

$$a_n = a_n(\infty) + \Delta a_n. \tag{6}$$

The first term on the right-hand side of each equation gives the steady-state value of the variable, which is assumed to be independent of position, and the second term gives the deviation from this value. Substitution of Eqs. 5 and 6 into Eq. 4 gives

$$\partial\Delta[S]/\partial t + \sum_{n=0}^{N} [B_n]\partial\Delta a_n/\partial t = D_S\nabla^2\Delta[S] + \sum_{n=1}^{N} D_n[B_n]\nabla^2\Delta a_n. \tag{7}$$

If the changes in $[S]$ are small, Δa_n is expected to be proportional to $\Delta[S]$,

$$\Delta a_n = \alpha_n \Delta[S], \tag{8}$$

in which α_n is a proportionality constant, $0 \leq n \leq N$. In the case of 1:1 binding of S to B_n, it is easy to show that $\alpha_n = K_{D,n}/([S] + K_{D,n})^2$, in which $K_{D,n}$ represents the dissociation constant of SB_n; if $[S] \ll K_{D,n}$, $\alpha_n \approx 1/K_{D,n}$. The assumption of direct proportionality between Δa_n and $\Delta[S]$, Eq. 8, is similar to the assumption of direct proportionality between a_n and $[H^+]$ that was used by Junge and McLaughlin (1987). Incorporation of Eq. 8 into Eq. 7 gives

$$\partial\Delta[S]/\partial t = D_S^{app}\nabla^2\Delta[S], \tag{9}$$

in which D_S^{app} is given by

$$D_S^{app} = \frac{D_S + \sum_{n=1}^{N} D_n\alpha_n[B_n]}{1 + \sum_{n=0}^{N} \alpha_n[B_n]}. \tag{10}$$

In some cases, such as proton diffusion in muscle (see below), the first terms in the numerator and denominator of Eq. 10 are negligible compared with the second terms, so that Eq. 10 can be written

$$D_S^{app} = \frac{\sum_{n=1}^{N} D_n\alpha_n[B_n]}{\sum_{n=0}^{N}\alpha_n[B_n]}. \tag{11}$$

In such cases, D_S^{app} depends only on the relative concentrations of the buffers and their diffusion constants. It does not depend on D_S.

In the case of proton diffusion, it is useful to relate the terms $\alpha_n[B_n]$ in Eqs. 10 and 11 to the buffering power, β_n, contributed by buffer species n (defined to be a positive

number, see Eqs. 36 and 38 in Roos and Boron, 1981),

$$\beta_n = -\Delta[H_n]/\Delta pH \quad (12)$$

$$= (\Delta[H_n]/\Delta[H^+])(-\Delta[H^+]/\Delta pH) \quad (13)$$

$$= (\alpha_n[B_n])\{[H^+]\ln(10)\}. \quad (14)$$

Eq. 10 becomes

$$D_H^{app} = \frac{D_H[H^+]\ln(10) + \sum_{n=1}^{N} D_n\beta_n}{[H^+]\ln(10) + \sum_{n=0}^{N} \beta_n}, \quad (15)$$

and Eq. 11 becomes

$$D_H^{app} = \frac{\sum_{n=1}^{N} D_n\beta_n}{\sum_{n=0}^{N} \beta_n} \quad (16)$$

$$= D_B^{av} \frac{\beta_{mob}}{\beta_{mob} + \beta_{immob}}. \quad (17)$$

D_B^{av}, the weighted average diffusion constant of the mobile buffers, is given by

$$D_B^{av} = \frac{\sum_{n=1}^{N} D_n\beta_n}{\sum_{n=1}^{N} \beta_n}; \quad (18)$$

β_{mob}, the total buffering power of the mobile buffers, is given by

$$\beta_{mob} = \sum_{n=1}^{N} \beta_n; \quad (19)$$

and β_{immob}, the buffering power of the immobile buffers, is given by

$$\beta_{immob} = \beta_0. \quad (20)$$

The expressions for D_H^{app} given above are essentially the same as those given in Junge and McLaughlin (1987). Consequently, the same expression for D_H^{app} is obtained if either (*a*) the pH is considerably greater than the largest pK of the buffers, in which case there is no other restriction on the magnitude of the pH gradient (Junge and McLaughlin, 1987), or (*b*) the pH gradient is small, in which case there is no restriction on the pK's of the buffers (this article).

Numerical example: proton diffusion in skeletal muscle

After action potential stimulation of frog skeletal muscle, there is a rapid increase in myoplasmic pH. Baylor et al. (1982, 1987), using intact muscle fibers injected with the pH indicator phenol red, estimated the amplitude of this increase to be 0.003–0.004 pH units at 16°C. Similar changes in myoplasmic pH have been observed in cut muscle fibers containing either phenol red or 4′,5′-dimethyl-5-(and-6-)carboxyfluorescein (Me_2CF) (Irving et al., 1989). In these experiments, the signal recorded with Me_2CF was consistently earlier and briefer than that recorded with phenol red. At 18°C, the Me_2CF pH signal had a time to half-peak, following that of the action potential, of ~10 ms (compared with ~20 ms with phenol red) and a half-width of ~50 ms (compared with ~100 ms with phenol red). The slower time course of the phenol red signal is surprising because the reaction between protons and this indicator appears to be rapid, with a delay of <10 μs in a temperature jump experiment at pH 7.4 at 25°C (Fig. 4.11 *A* in Hammes, 1974).

A possible explanation for the different time courses of the two pH signals is that phenol red and Me_2CF become bound to different sarcomeric sites that are located within a transient pH gradient after stimulation. (A large fraction 0.7–0.8 of these indicators is bound or sequestered in some manner inside cut fibers, although the binding locations are unknown [Irving et al., 1989]). If the proton flux out of the myoplasmic compartment after stimulation were spatially localized within a sarcomere, a transient pH gradient could develop in the same way that a gradient in free [Ca] may be produced by localized sarcoplasmic reticulum Ca release (Cannell and Allen, 1984). Indeed, the early increase in myoplasmic pH after stimulation may be due to protons entering the sarcoplasmic reticulum (Baylor et al., 1982, 1987). After the proton flux ceases, the characteristic duration of the pH gradient (t) is expected to be related to its spatial extent (x) by the Einstein relation, $x^2/t = 2D_H^{app}$. If the proton flux out of myoplasm were located closer to the hypothetical binding sites for Me_2CF than to those for phenol red, the Me_2CF pH signal would be earlier and briefer than the phenol red signal, as observed, and have a larger peak amplitude.

The feasibility of this hypothesis can be tested with the theory developed above. Intact muscle fibers contain both mobile and immobile pH buffers. Most of the mobile buffering power is provided by carnosine (Godt and Maughan, 1988), whereas the immobile buffering power would be expected to be provided by structures such as the thick and thin filaments and the sarcoplasmic reticulum. In cut muscle fibers, the intrinsic mobile buffers are progressively lost from the ends of the fibers. Over a single sarcomere, however, the buffer concentration is effectively constant over the time scale of interest, as assumed in the Theory section. Because some of the myoplasmic buffers have pK's near the value of myoplasmic pH (Godt and Maughan, 1988), the derivation of

Junge and McLaughlin (1987) does not apply to proton diffusion in muscle. The theory presented above does apply, however, because the pH changes associated with action potential stimulation are small.

Experimental estimates of the buffering power of intact frog muscle fibers vary from 26 to 38 mM/pH unit at room temperature (20–26°C), with an average value of 33 mM/pH unit (Bolton and Vaughan-Jones, 1977; Abercrombie et al., 1983; Curtin, 1986). These estimates are based on measurements of internal pH obtained with a time resolution of some tens of seconds. Consequently, they may include contributions from slowly reacting buffers or pH-regulating systems that would have little effect on an active pH transient that develops in tens of milliseconds. Thus, the average value of 33 mM/pH unit is likely to be an upper limit of the buffering power available during the early pH transient after action potential stimulation.

Of the 33 mM/pH unit of total buffering power, ~12 mM/pH unit is expected to be associated with mobile buffers (calculated from the data in Table 4, pH = 6.8–7.18, in Godt and Maughan, 1988) and the difference, 33 − 12 = 21 mM/pH unit, is expected to be associated with immobile buffers. These values, 12 and 21 mM/pH unit, are several orders of magnitude larger than the value of $[H^+] \ln(10)$, which is usually <1 μM. Consequently, the term $[H^+] \ln(10)$ in the denominator of Eq. 15 can be ignored. The term $D_H[H^+] \ln(10)$ in the numerator of Eq. 15 can also be ignored, because the value of D_H is 8×10^{-5} cm^2/s (in a dilute aqueous solution at 18°C, Robinson and Stokes, 1959) and that of D_B^{av} (Eq. 18) is estimated to be 5×10^{-6} cm^2/s (see next paragraph). Thus, Eqs. 16–20 can be used to evaluate D_H^{app}.

According to Godt and Maughan (1988), carnosine (β-alanylhistidine) accounts for 90% of the mobile buffering power of frog myoplasm at pH 7. Consequently, the terms for carnosine dominate the numerator and denominator of Eq. 18 and D_B^{av} is approximately equal to the diffusion constant of carnosine. Carnosine is similar in size to glycylleucine and leucylglycine and should have a similar diffusion constant, 6×10^{-6} cm^2/s in aqueous solution at 25°C (Longsworth, 1953). At 18°C, the average temperature of our experiments, its diffusion constant should be $\sim 5 \times 10^{-6}$ cm^2/s. Within a single sarcomere, the value of the diffusion constant is expected to lie between that for free solution and that for diffusion along a fiber, from sarcomere to sarcomere, which for many small molecules is about half the value in free solution (Kushmerick and Podolsky, 1969). Hence, D_B^{av} for diffusion within a sarcomere is expected to lie within the range 2.5–5 $\times 10^{-6}$ cm^2/s. With Eq. 17 and the above estimates for β_{mob}(12 mM/pH unit) and β_{immob}(21 mM/pH unit), D_H^{app} in intact frog twitch fibers is expected to be (12/33) $\times$ 2.5–5 $\times 10^{-6}$ cm^2/s = 1–2 $\times 10^{-6}$ cm^2/s at 18°C.

The Einstein relation and this value of D_H^{app} indicate that, if a pH gradient with a spatial extent of 1 μm were set up transiently within each half sarcomere, it would be expected to decay in 2.5–5 ms. In cut fibers, however, the mobile buffers in the myoplasm should equilibrate with those in the end-pool solutions, which, in many previously reported experiments, had a buffering power that was only a small fraction of that estimated for the mobile buffers in intact muscle. For example, Irving et al. (1989) used an end-pool solution that had an estimated buffering power of 3 mM/pH unit at pH 7, of which ~90% was contributed by 5 mM Pipes. The diffusion constant of Pipes (302 mol wt) is expected to be similar to that of carnosine (226 mol wt), so that D_H^{app} in a cut fiber after equilibration with the end-pool solution is expected to be (3/24) $\times$ 2.5–5 $\times 10^{-6}$ cm^2/s = 0.3–0.6 $\times 10^{-6}$ cm^2/s at 18°C. In this circumstance, it is possible that, after an action potential, a pH gradient within 1 μm could last 10 ms or longer. This duration is sufficiently long to be consistent with the idea that the different time courses of the phenol red and Me_2CF pH signals in cut fibers might arise from a myoplasmic pH gradient and indicator binding to different locations within this gradient (Irving et al., 1989).

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